

Molecular studies of human immune recognition of allergens

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Allergy provides a particularly good model for studying the genetics of human immune responsiveness and its relationship to disease, because one can establish a clear *causal* relationship between specific IgE antibody (Ab) responses and the expression of specific allergies (3). There is evidence for at least two types of genetic control of IgE responsiveness in humans, namely, HLA-linked control of specific Ab responses and non-HLA-linked control of the overall production of IgE, where high IgE is inherited as a recessive trait (3). We have previously reported significant associations between particular HLA types and specific IgE (and IgG) Ab responses to several highly purified allergens (Table 1; ref. 8). In particular, the associations between HLA-DR2/Dw2 and responsiveness to the *Amb* V (Ra5) homologues, *Amb* a V ($M_r = 5000$) and *Amb* t V ($M_r = 4400$), from *Ambrosia artemisiifolia* (short) and *A. trifida* (giant) ragweed pollens, are noteworthy. For example, 95% of Caucasoid subjects who had IgE Ab to *Amb* a V following natural pollen exposure possessed the DR2-associated MLR specificity, Dw2 (4); and *all* Dw2+ subjects who were immunized with short ragweed pollen extracts made IgG Ab to *Amb* a V (5). The molecular basis of specific recognition of three *Amb* V homologues has now been investigated in a *prospective* immunotherapeutic study using ragweed extracts. Molecular biological techniques have been used to study the HLA-D DNA sequences of both *Amb* V responders and non-responders.

IgG Ab responses toward *Amb* t V, *Amb* p V (from *A. psilostachya*, western ragweed) and *Amb* a V were studied in 12 matched pairs of HLA-

DR2+ and DR2- Caucasoid ragweed-allergic patients (plus 6 additional DR2-unmatched patients) following *sequential* allergen immunotherapy with giant, western and short ragweed extracts containing known amounts of the three *Amb* V Ags (7; Marsh et al, in preparation). Serum was taken for RIA of specific Abs, before and several times after increasing dosages (0.033–11 µg) of each *Amb* V species. Additional HLA-D typing, by DNA Restriction Fragment Length Polymorphism (RFLP) mapping, allowed us to define which DR2+ subjects had RFLP patterns corresponding to the Dw2 subtype (Zwollo, P., Ansari, A. A. and Marsh, D. G., submitted for publication).

After Phase I of the study, 8/12 DR2+ subjects made IgG Ab responses to *Amb* t V, whereas only 4/12 matched DR2- subjects made (low) levels of IgG Ab. The Ab levels were significantly higher in the DR2+ than the DR2- group ($P = 0.01$). After the second phase, 8/10 DR2+ subjects had IgG Ab to *Amb* p V, whereas all 10 matched (and 6 unmatched DR2-) subjects were IgG Ab- (Ab levels; $P \leq 0.01$). All 6 DR2+ subjects completing Phase III were IgG Ab+, but only one of 15 DR2-subjects were IgG Ab+ (Ab levels; $P < 0.001$). Additional statistical analyses showed that Dw2 was more strongly associated with responsiveness to the *Amb* V Ags than DR2. These data confirm the previously observed DR2/Dw2 associations with immune responsiveness to *Amb* t V and *Amb* a V, and extend these findings to include *Amb* p V. Furthermore, *concordant* responsiveness toward all three *Amb* V species was invariably found in persons possessing DR2/Dw2 (*cf* earlier data for *Amb* a V and *Amb*

t V obtained by Goodfriend, Marsh and their associates; discussed in ref. 6).

The genetic alleles representing the HLA-D subtypes DR2.2 (α and β genes) and DQw1.2 (α and β genes) are in “linkage disequilibrium” (usually found together on the same chromosome) in Caucasoid populations, and encode DR and DQ Ia molecules that are both believed to contribute to the Dw2 MLR specificity. Thus, it is not possible to discriminate between whether DR2.2 or DQw1.2 is the Ia molecule usually associated with immune responsiveness to the *Amb* V allergens. Furthermore, it is generally believed that the hypervariable regions (HVRs) of the second exons of HLA-DR β , DQ α and DQ β encode the antigen-binding portions of the class II (HLA-D) molecules (1). Investigation of the DNA sequences of these HVRs in a large number of typical and atypical *Amb* V responders and non-responders is therefore appropriate; moreover, this approach is now *feasible* with the advent of revolutionary new gene amplification technology, the Polymerase Chain Reaction (PCR; reviewed in Ref. 2).

The PCR was used to amplify (by $\sim 10^6$ -fold) the DR β DQ α and DQ β second-exon segments from leukocyte genomic DNA from up to 40 patients at a time within a 3-4 hr. period, without the need to prepare and screen genomic or cDNA libraries (10 and Zwolo et al., in preparation). (This involves amplifying segments of ~ 300 bps from the $\sim 3 \times 10^9$ bps in the human genome!). The DR β and DQ β segments of certain patients (especially exceptional *Amb* a V + /Dw2- subjects) were purified, cloned into M13, and sequenced by the dideoxy method. For rapid screening of larger numbers of subjects, sequence-specific oligonucleotides (SSOs) were used in dot-blot experiments (2, 9) to investigate the presence or absence of specific HVRs found in certain DR β , DQ α and DQ β subtypes (especially DR β 2.2, DQ α 1.2 and DQ β 1.2). Preliminary data suggest that either amino acids positions 67-71 of DR β or 26-30 of DQ β are implicated in specific immune responsiveness to the *Amb* V Ags.

The location of Ia/T-cell determinants on the *Amb* V molecules can be inferred from the known amino acid sequences of *Amb* V and *Amb*

Table 1

Non-parametric statistical analysis of HLA associations with specific Ab responsiveness toward some ultra-pure and highly purified pollen allergens in Caucasoid subjects

Systematic name	Old name	Rel. molr. mass, M _r	Primary association	P values*		
				1st study	2nd study	overall
<i>Ambrosia artemisiifolia</i> and <i>A. trifida</i> (short and giant ragweeds)						
<i>Amb a</i> I**	AgE	37,800	none	–	ND	–
<i>Amb a</i> III	Ra3	12,300	A2	4 × 10 ⁻²	NS	–
<i>Amb a</i> V	Ra5	5,000	DR2/Dw2	2 × 10 ^{-5***}	4 × 10 ^{-10***}	< 10 ^{-9***}
<i>Amb a</i> VI	Ra6	11,500	DR5	7 × 10 ⁻⁷	1 × 10 ⁻³	< 10 ⁻⁶
<i>Amb t</i> V	Ra5G	4,400	DR2/Dw2	1 × 10 ^{-2***}	3 × 10 ^{-2***}	< 10 ^{-3***}
<i>Lolium perenne</i> (perennial rye)						
<i>Lol p</i> I**	Rye I	27,000	DR3	1 × 10 ⁻²	6 × 10 ⁻³	< 10 ⁻³
<i>Lol p</i> II	Rye II	11,000	DR3	4 × 10 ⁻²	7 × 10 ⁻³	10 ⁻³
<i>Lol p</i> III	Rye III	11,000	DR3	7 × 10 ⁻³	1 × 10 ⁻⁵	< 10 ⁻⁵
<i>Lol p</i> X	Cytochrome	12,000	none	–	–	–

*P values are studies of Ab responses resulting primarily from natural allergenic exposure. IgE Ab data were used in most cases; IgE-mediated skin-test data were used for *Amb* a I and *Amb* a III and IgG Ab data were used where there were an insufficient number of IgE Ab + subjects (*Amb* t V and *Lol* p X). Two-tail Fisher's Exact Tests have been used for analysis of the first study and one-tail tests for the second study.

**Highly purified allergens ($\geq 97\%$ pure); all other allergen preparations estimated to be $\geq 99.5\%$ pure.

***The stronger associations (for which P-values are cited) were found with the Dw2 MLR-defined specificity found in *ca* 90% of DR2 + Caucasoid individuals.

t V, which show high degrees of homology at positions 17-26 and 31-35, yet are virtually non-cross-reactive (6). These two regions are probably linked by a disulphide bond and are also amphipathic. Further information regarding the structure of this putative Ia/T-cell determinant comes from a comparison of the sequences of the *Amb* V molecules with those of the wheat germ agglutinin domains, which exhibit significant homology, especially in regard to the positions of their half-cystine residues (6).

Further analysis of the sequences of the Ia molecules and Ia/T-cell determinants involved in the recognition of the *Amb* V molecules and, eventually, of the T-cell receptors should lead to a better understanding of the molecular basis of immune recognition in humans.

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